conditions of TDO patients, our results support the hypothesis that Dlx3 is an essential regulator for development of hair follicle.

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Program/Abstract # 433
Molecular consequences of a frameshifted Dlx3 mutant leading to Tricho-Dento-Osseous syndrome

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The homeodomain protein Distal-less-3 (Dlx3) plays a crucial role during embryonic development. In humans, a frameshift mutation in the coding sequence of the DLX3 gene results in an ectodermal dysplasia called Tricho-Dento-Osseous syndrome (TDO). The main features of this autosomal dominant disorder are defects in hair, teeth and bone. To investigate the functional alterations caused by the mutated $Dlx3^{TDO}$ isoform ex vivo, we used tetracycline-inducible cell lines in which the expression of $Dlx3^{WT}$ and/or $Dlx3^{TDO}$ could be regulated. Immunocytochemical analysis revealed that both $Dlx3^{WT}$ and $Dlx3^{TDO}$ recombinant proteins are targeted to the nucleus. However, as demonstrated by Electrophoresis Mobility Shift Assay, $Dlx3^{TDO}$ is not able to bind to the canonical Dlx3 binding site. Furthermore, we demonstrate that the frameshifted C-terminal domain in $Dlx3^{TDO}$ is responsible for the loss of DNA binding activity since the C-terminal domain in $Dlx3^{WT}$ is not required for DNA binding activity. Although $Dlx3^{TDO}$ cannot bind to Dlx3 responsive element it can interact with $Dlx3^{WT}$. Reporter assays showed that $Dlx3^{TDO}$ has a defective transcriptional activity. Moreover, the transcriptional activity of $Dlx3^{WT}$ is significantly reduced in the presence of the mutated isoform. Taken together, these data demonstrate that many of the developmental defects associated with TDO are potentially a consequence of the dominant negative effect of the $Dlx3^{TDO}$ protein on its wild type counterpart.

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Program/Abstract # 434
Role of T and Tbx6 in mesodermal patterning

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Tbx6 and Brachyury (T), two T-box transcription factors, are co-expressed in the primitive streak of the developing mouse embryo and are essential for mesodermal patterning. Tbx6 has an additional expression domain in the presomitic mesoderm independent of T, and T is expressed in the node and notochord independent of Tbx6. The T-box proteins are related through a conserved T-box DNA binding domain, and accordingly, Tbx6 can bind T's consensus binding sequence in vitro. We are further investigating how T and Tbx6 work together and independent of each other to activate common and/or different downstream targets and specify different cellular and morphological properties. Results from these studies will give further insight into how T and Tbx6 function in primitive streak and paraxial mesoderm formation.

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Program/Abstract # 435
The identity and fate of Tbx4-expressing cells reveal previously unknown developmental decisions in the allantois, limb, and proctodeum

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The T-box gene *Tbx4* is critical for the formation of the umbilical vessels as well as for the initiation and proper morphogenesis of the hindlimb. Previous work has shown that it is expressed in broad domains throughout the allantois and the hindlimb, as well as in the lung and proctodeum. We have examined the expression of *Tbx4* in greater detail and used a cre-mediated lineage reporter to examine the eventual fates of cells that express *Tbx4*. Despite the observation that loss of *Tbx4* produces profound defects in the developing allantois vasculature, the presumptive endothelial cells of the allantois do not appear to express *Tbx4*, and lineage trace analysis reveals that much of the umbilical endothelium has never expressed *Tbx4*. These results imply that endothelial and non-endothelial lineages are segregated well before the onset of vasculogenic genes such as *Flk-1*, and also demonstrate a novel role for the peri-vascular tissue in the development of continuous vascular structures. Likewise, examination of the relationship between the expression of *Tbx4* in the posterior mesenchyme and the eventual fate of *Tbx4*-expressing cells suggests that various distinct appendages such as the allantois, hindlimb, and external genital all arise from a single contiguous domain. In addition, although *Tbx4* is normally associated with the hindlimb, we have found and characterized two domains of expression in the forelimb which produce cells that segregate to specific regions of the forelimb.

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Program/Abstract # 436
Ash2l: A Novel interacting cofactor of DiGeorge syndrome transcription factor Tbx1

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DiGeorge syndrome (DGS) is a common syndrome associated with 22q11 deletions. Most patients with DGS are born with severe heart defects. Congenital heart disease is the most commonly occurring birth defect and relatively little is known about the molecular basis of these defects. Mouse models have implicated Tbx1 as a critical gene within the commonly deleted region. Tbx1 encodes a nuclear transcription factor that binds DNA and regulates downstream genes. Tbx1 direct targets and its transcriptional complex are largely unknown. We have identified a potential transcriptional cofactor, Ash2l. Ash2l is known to be part of a histone methyltransferase complex involved in epigenetic transcriptional regulation. Two non-overlapping interacting Ash2l domains were independently found to interact with Tbx1 in our unbiased yeast two-hybrid screen. These interactions were confirmed in mammalian cells. Ash2l mRNA and protein is widely expressed in the mid-gestation mouse embryo, including in Tbx1 expression domains. While Ash2l+/− mice are normal, complete loss of Ash2l is lethal early in embryogenesis.Ash2l physically interacts with Tbx1. Very early embryonic lethality of Ash2l null mice suggests this protein is critically

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Program/Abstract # 437
Processing of Lunatic fringe protein by subtilisin/furin-like proprotein convertases contributes to its short intracellular half-life
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During vertebrate segmentation, oscillatory activation of Notch1 signaling is important in the clock that regulates the timing of somitogenesis. In mice, the cyclic activation of Notch1 requires the periodic expression of Lunatic fringe (*Lfng*). For LFNG to play a role in the segmentation clock, its cyclic transcription must be coupled with post-translational mechanisms that confer a short protein half-life. LFNG protein is cleaved and released into the extracellular space. We hypothesize that this secretion contributes to a short LFNG intracellular half-life, facilitating rapid oscillations within the segmentation clock. To test this hypothesis, we localized N-terminal protein sequences that control the secretory behavior of fringe proteins. We find that LFNG processing is promoted by specific pro-protein convertases including furin and SPC6. Mutations that alter LFNG processing do not prevent its secretion, but do alter its intracellular half-life. These mutations do not affect LFNG function in the Notch pathway, thus protein half-life affects the duration, but not the specificity of LFNG activity. Targeted mutation has been used to express Golgi-tethered LFNG from the endogenous locus allowing us to examine the *in vivo* effects of altered LFNG processing on oscillatory Notch signaling in the segmentation clock. These results have important implications for the mechanisms that contribute to the tight control of Notch signaling during vertebrate segmentation.

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Program/Abstract # 438
Evidence for *Hox*-specified positional identities in adult vasculature
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The role of *Hox* transcriptional regulators in establishing positional identities during embryonic patterning is well documented; however, the activity of this conserved gene family in adult tissues is only recently being elucidated. The existence of positional information within the cardiovascular system in particular remains poorly studied. The paucity of information regarding *Hox* gene activity in vascular tissues, including in vivo expression data—a prerequisite for defining functional roles, leaves much to be discovered and offers great potential for significant advancements in our understanding of cardiovascular disease mechanisms. To gain insight into the global vascular activity of *Hox* proteins we selected several genes for *lacZ*-based transgenic mouse reporter gene analysis: *Hoxc10* and *Hoxc11*, which harbor posteriorly restricted embryonic expression patterns, and *Hoxa3*, whose expression is more anterior. In this study we show that these genes exhibit zonal vascular patterns that are reminiscent of the distinct A–P activity domains found during embryonic patterning. Medial, vascular smooth muscle cells (VSMCs) that originate from the same site have the potential for differential responses to various stimuli (hemodynamic stress, hypoxia, cell signaling, etc.); the molecular determinants specifying these cell phenotypes are currently obscure. *Hox* activity profiles (*Hox* code) might pre-determine these response options—an idea consistent with the original concept of *Hox* genes acting as selector genes that establish compartment-specific differentiation pathways in the embryo.

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Program/Abstract # 439
Only posterior interdigit provides positional information to its anterior PFR to specify each digit identity
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The zone of polarizing activity is the primary signaling center controlling anterior posterior patterning of the amniote limb bud. The autopodial interdigits (IDs) are secondary signaling centers proposed to specify digit identity, through an as yet unidentified signal or signals. Here we focus on the digit and define a region of the digital ray that we name the phalanx-forming region (PFR) that expresses Sox9, Bmpr1b, and is phosphorylated-SMAD1/5/8 positive. The PFR cells are committed to the cartilage lineage, then respond unidirectionally to ID signals and finally are incorporated into the digit primordium. Using a novel in vivo reporter assay, we establish that each PFR has a unique SMAD1/5/8 activity, developing in a spatially- and temporally-restricted manner; this activity correlates with digit identity. Using our data, we propose a model that incorporates data from human, mouse, and chick, and provides a mechanism for understanding formation and variation of digits (number, size, and shape of phalanges) among amniotes, as well as a mechanistic explanation for human defects such as brachydactyly type A2. In this conference, we will show detail in which how only posterior interdigit provides positional information to its anterior PFR to specify each digit identity.

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Program/Abstract # 440
Sonic Hedgehog signaling in the apical ectodermal ridge is essential for proper patterning of the vertebrate limb
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Sonic Hedgehog (*Shh*) in the developing limb has been shown to mediate the action of the Zone of Polarizing Activity (ZPA). Through the analysis of *Shh* target genes, for example *Ptc1* and *Gli1*, it is known that the *Shh* signaling cascade is active in the limb mesoderm. Recently, array-based experiments have found that a number of target genes of the *Shh* signaling pathway were also present in the limb apical ectodermal ridge (AER). To investigate the possibility of *Shh* signaling in the AER, SHH protein was immunolocalized in the limb bud ectoderm including the apex. A *Ptc1LacZ* knock-in mouse was used to detect *Ptc1* in the posterior AER of the developing limb. To determine if *Shh* signaling in the AER plays a role in limb patterning,